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Dmitriew, Caitlin ; Blows, M W ; Rowe, L

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Ontogenetic Change in Genetic Variance in Size Depends on Growth Environment

C. Dmitriew,[1,*] M. W. Blows,[2] and L. Rowe[1]

[1]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario M5S 3B2, Canada; 2. School of Biological Sciences, University of Queensland, St. Lucia, Queensland 4072, Australia

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Keywords: compensatory growth, quantitative genetics, ontogeny, G × E, canalization, mutational target size.

Introduction

Variation is a ubiquitous property of growth (a change in size) during ontogeny, which in turn leads to variance in age and size at maturity, two traits that are central to shaping life history and, consequently, fitness (Roff 1992, 2000; Stearns 1992; Charlesworth 1994; Arendt 1997). As with any phenotypic character, genetic, maternal, and environmental variance may contribute to the abundant variance observed in growth (Atchley 1984; Riska et al. 1984; Atchley and Zhu 1997). Both phenotypic and genetic variance in some traits are known to change over the ontogeny of single cohorts, and these changes, when they occur during the juvenile stage, are in some cases linked to growth. Given the strong dependence of fitness on size at maturity, one might expect that selection would cause this phenotypic variance in size to decline as maturation is approached.

Early researchers have demonstrated exactly this pattern, which has been referred to as targeted (Tanner 1963), compensatory (Monteiro and Falconer 1966), or convergent (Riska et al. 1984) growth. These early studies were usually unable to determine the extent to which this reflected a contraction of genetic or environmental variance in size. Subsequent phenotypic manipulations have shown that environmentally induced compensatory growth is common to a variety of taxa (Metcalfe and Monaghan 2001; Ali et al. 2003; Mangel and Munch 2005). Typically in these studies, growth is briefly reduced relative to a control group with experimental diet restriction. When food supply is then returned to control levels, individuals who experienced this transient reduction in growth tend to accelerate growth beyond that observed in the controls, thereby reducing variance in size by the time maturity is reached (Metcalfe and Monaghan 2001; Ali et al. 2003).

In some cases, ontogenetic changes in genetic variance in size may parallel those seen in phenotypic studies of growth. Early laboratory studies of prereproductive growth in rodents detected a substantial contraction in the additive genetic variance (V_a) of size as maturation was approached (Cheverud et al. 1983; Atchley 1984; Riska et al. 1984). More recently, estimates of V_a in wild and domestic animals have also begun to detect changes in the V_a of size over juvenile ontogeny (e.g., Réale et al. 1999; Wilson et al. 2005; Wilson and Réale 2006). However, these results from wild populations are not always consistent with the early laboratory studies. More broadly, ontogenetic changes in the genetic variance of a variety of traits other than size do not suggest a single temporal pattern: variance may increase, decrease, or change as a combination of the two in sequence (e.g., Promislow et al. 1996; Tatar et al. 1996; Hughes et al. 2002; Snoke and Promislow 2003;

* Corresponding author; e-mail: c.dmitriew@utoronto.ca.

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Charmantier and Garant 2005; Charmantier et al. 2006; Wilson and Réale 2006).

Likewise, theory does not suggest that we should expect a single ontogenetic pattern in genetic variance, with alternatives emphasizing either selection or mutation. A history of strong selection on size at maturity may have led to the evolution of convergent growth and canalization of variance in size as maturation is approached (Riska et al. 1984). Canalized traits are relatively insensitive to environmental or genetic perturbations as a consequence of the action of buffering mechanisms (Stearns and Kawecki 1994; Dworkin 2005; Flatt 2005). This hypothesis can account for a contraction in both environmental and genetic variance at maturity.

Alternatively, according to the theory of mutation accumulation (Medawar 1952), genetic variance in traits is expected to increase over time as the ability of selection to purge mutations declines with age. Houle (1998) has also argued that traits expressed later in ontogeny effectively have a larger mutation target size, because genes expressed early in life can affect the expression of all subsequently expressed genes (variance compounding). A similar argument could be mounted for the compounding of environmental variance (Price and Schluter 1991). This variance-compounding hypothesis would suggest that genetic variance in size may actually expand as maturation is approached, an effect that could moderate or even reverse the selection argument (above). Finally, the environment in which growth occurs might mediate how V_a changes over ontogeny. For example, exposure to stress or novel environments might reveal genetic variance that is hidden in more benign conditions (cryptic genetic variance), although the data addressing this hypothesis are mixed (Hoffman and Parsons 1991; Hoffmann and Merilä 1999; Charmantier and Garant 2005; Flatt 2005). Environmentally induced stress in the wild may account for the disconnect between laboratory and field studies of the ontogeny of size V_a.

In this article, we compare the ontogeny of environmentally and genetically generated variance in the size of growing ladybird beetles *Harmonia axyridis*. We first experimentally induce variance in body size by employing a typical compensatory growth experiment (Metcalfe and Monaghan 2001; Ali et al. 2003). In the control group, larvae are fed at a constant high ration, whereas in the low-food (compensating) treatment, larvae are fed for a brief period at a low food level and then returned to the control food level until maturation. There is some evidence of a cost to fitness of this low-food regime (Dmitriew and Rowe 2007), and thus the low-food treatment may be considered a stress. This environmental experiment is crossed with a half-sib design (Falconer and MacKay 1996), which allows us to determine whether genetic variance in size expands or contracts over ontogeny and whether this effect depends on the growth conditions experienced.

The parallel literatures on the ontogeny of genetic variance in size (e.g., Cheverud et al. 1983; Atchley 1984; Riska et al. 1984) and that of compensatory growth induced by environmental manipulations (Metcalfe and Monaghan 2001; Ali et al. 2003) have not been well integrated, and our design allows us to take a step in this direction. We know of no studies that examined genetic variance in compensatory growth, although some studies suggest that it is present among populations (e.g., Fraser et al. 2007). It is also unknown whether fast-growing genotypes in normal growth conditions are the same ones that grow fast when stressed. The possibility exists that genes involved in compensatory growth are not those involved in normal growth, which would be manifested as a strong genotype × environment (G × E) effect. Such interactions have not been investigated in the context of compensatory growth, and their presence may account for some of the genetic variance in growth that appears to be widespread (Arendt 1997; Roff 2000).

Methods

Ladybird beetles *Harmonia axyridis* (Coleoptera: Coccinellidae) were collected in June 2007 on the University of Toronto campus and placed individually in 10-cm-diameter petri dishes. Only final-instar larvae and pupae were collected to ensure that all individuals were unmated before the experiment. Beetles were fed a diet of irradiated *Ephestia kuehniella* eggs (Beneficial Insectary, Redding, CA) and pea aphids (*Acyrthosiphon pisum*) until June 27, when all beetles had eclosed to adults. Beetles were then paired, and matings were conducted according to a half-sib design (Falconer and MacKay 1996). On the first day, 40 males were randomly selected and each paired with a single randomly selected virgin female. Males observed to copulate successfully within 4 h were then remated to a second randomly selected virgin female the following day. Females were isolated following mating and allowed to oviposit. We used larvae hatched from eggs over a 4-day period, which was necessary to obtain a sufficient number of offspring per family.

Twenty-one sires produced a sufficient number of offspring to be included in the experiment. Thus, we had a total of 42 dams. Most families included a total of 20 hatchlings (10 allocated to each feeding treatment), although three families had a reduced initial size ranging from 12 to 16 offspring. In total, we began with 822 larvae distributed between two feeding treatments. These larvae were held individually in petri dishes containing a piece of cotton soaked in sugar solution (1 tbsp organic cane

sugar/100 mL). In the high-food treatment, larvae were fed daily from hatching to eclosion. In the low-food treatment, larvae were fed a similar ration but for the first 6 days after hatching were fed only every second day. Larvae were initially provided with early-instar aphids, and prey size was gradually increased over the first 6 days starting with 4 aphids per day and increased by 2 every second day. Natural populations of *H. axyridis* face considerable variation in resource availability since their primary food source, aphids, are highly ephemeral. From day 7 on, which is within a day of molt to final instar, both treatments received rations of 10–12 aphids daily, with a mean individual live mass of 200 ± 20 mg.

Each larva was measured on two occasions. Wet mass was recorded on day 7 before the daily feeding and again within 24 h of eclosion to adult. Pronotum width was measured on the day of the molt into the final (fourth) instar, which generally occurred within a day or two of the mass measurements (day 7), depending on the treatment, and again after eclosion. This was done to allow consistent measurement using landmarks, as the pronotum does not change in size within an instar, although swelling of the body beneath the plate occurs during growth.

Phenotypic Analysis: Larval Growth and Survival

We began by analyzing the mean phenotypes to establish whether our food manipulation had created significant differences in size midway through ontogeny and whether compensatory growth occurred late in ontogeny. As an indicator of the extent of stress induced by our low-food manipulation, we assessed mortality rates for juveniles during the early (day 1–7) and late (day 7 to adult) periods of ontogeny. These correspond to the periods of low and control food levels in our low-food treatment. Initial analyses showed that both pronotum width and mass were highly correlated and responded to the food manipulation in a similar way (as was previously demonstrated in this species; e.g., Dmitriew and Rowe 2007). Thus, a composite measure of body size was calculated using a principal component (PC) analysis of ln-transformed mass and pronotum width at each measurement period, and PC1 (body size) captured 92.05% percent of the variance. We used two-way ANOVAS to determine the effect of feeding treatment and hatch day on larval (fourth-instar) body size and adult body size, development time, and growth rate of body size. In our analyses, late-hatched individuals tended to be significantly larger and quicker to develop than early-hatched individuals. We are not interested in exploring the effect of hatch day, but we have included it (and its interaction with treatment) in the models as a fixed factor to account for any variation attributable to this variable. Initial analyses of these data and genetic data suggested sex effects. Therefore, all analyses considered the sexes independently.

Genetic Variance in Size: Effects of Developmental Stage and Environment

For each individual, size measures (pronotum width and mass), taken at two points in ontogeny (see above) can be treated as a set of four age-specific traits across the two environments, resulting in eight traits that can be subjected to a single quantitative genetic analysis. Both measures of size (mass and pronotum width) were included in the analysis because although these traits are correlated, the precise allometric relationships between such traits in insects may depend on conditions experienced during development (e.g., Stevens et al. 1999; Strobbe and Stoks 2004; Dmitriew and Rowe 2005). Genetic variance over ontogeny in a variety of organisms also tends to depend on which size trait is measured (Atchley and Zhu 1997; Wilson et al. 2005).

Before genetic analysis, traits were standardized to a mean of 0 and a standard deviation of 1 for each measurement (mass or pronotum width) at each time period in order to control for differences in mean and variance due to traits being measured on different scales. Initial analyses suggested sex effects; therefore, in separate analyses for each sex, we estimated the two additive genetic 8 × 8 variance-covariance matrices using the mixed model

$$\mathbf{Y}_{ijkl} = \boldsymbol{\mu} + \mathbf{S}_i + \mathbf{D}_{j(i)} + \mathbf{H}_k + \boldsymbol{\epsilon}_{ijkl}, \quad (1)$$

where hatch day (**H**) was modeled as a fixed effect, and sire (**S**) and dam nested within sire (**D**) are treated as random effects. The residual variance was modeled in the matrix $\boldsymbol{\epsilon}$ in four blocks (one for each combination of food environment and time period), with an unconstrained covariance structure within each block (among size and mass). The genetic variance-covariance matrices (**G**) were estimated from the sire level by multiplying the variances and covariances in **S** by 4 (Falconer and MacKay 1996). Variance components were estimated using restricted maximum likelihood, and analyses were conducted in the SAS MIXED procedure (SAS Institute 2008). Since the traits were variance standardized before analysis, the genetic variances we report equate to narrow-sense heritabilities.

In order to test for the presence of genetic variance in trait combinations, a factor-analytic covariance structure was applied at the sire level (Kirkpatrick and Meyer 2004; Hine and Blows 2006). This approach takes the unstructured variance-covariance matrix at the sire level (the **G** matrix when multiplied by 4) and asks how many trait combinations (dimensions) are sufficient to explain the

Table 1: Additive genetic variance-covariance matrices for body size at two stages of ontogeny at high and low food

	High food				Low food			
	PW early	PW late	Mass early	Mass late	PW early	PW late	Mass early	Mass late
Males	.289	.136	.273	.124	.45	.291	−.005	.328
		.171	.136	.108	.211	.283	.132	.265
			.258	.12	.424	.285	.005	.317
Females	.563			.076	.192	.193	.061	.192
	.096	.016			.699	.453	−.007	.509
	−.082	−.014	.052			.492	.177	.481
	.153	.026	−.013	.044			.167	.134
	.069	.012	−.151	−.014	.498			.486
	−.083	−.014	−.109	−.051	.41	.373		
	−.06	−.01	.025	−.013	−.064	−.04	.013	
	−.016	−.003	−.117	−.032	.414	.36	−.047	.354

Note: PW = pronotum width.

estimated genetic variance contained in the **G** matrix. This is achieved by sequentially fitting fewer dimensions in each model, starting with the deletion of the trait combination that explains the least amount of genetic variance, then the second, and so on. A Cholesky factor representation of the dimensions is used to fit the reduced-rank covariance matrices. The significance of each factor is tested using a series of nested log-likelihood ratio tests. This statistic is calculated as −2 times the difference in log likelihood between hierarchically structured models, and the significance is tested using a χ^2 test with the degrees of freedom being the difference in number of parameters between successive models. The number of parameters here refers to the number of coefficients that are required to represent the factor in the lower Cholesky matrix (Hine and Blows 2006). In our case, we were estimating an 8 × 8 **G** matrix, and so the factor that explained the most genetic variance was represented by eight loadings in the lower Cholesky matrix, the second factor was represented by seven loadings, and so on. This allowed us to determine the number of genetic factors that explain a significant amount of genetic variance and, thus, the minimum number of factors that adequately describe the data. Eliminating factors that explain a negligible amount of variation reduces the number of unnecessary parameters and facilitates convergence of the model. After the number of significant genetic dimensions is identified, the reduced-rank **G** matrix that contains a reduced number of dimensions can be reconstructed using the factor loadings (Hine and Blows 2006, their eq. [6]). Given that bias may be introduced by constraining the model to a very small number of factors (tending toward overestimation of loadings on the major factors and underestimating of loadings on smaller factors), we follow the suggestion of Meyer and Kirkpatrick (2008) that the first nonsignificant genetic factor also be included in the analysis.

Hypothesis Test

The determination of the number of significant genetic factors underlying the genetic covariance among the eight traits facilitated the interpretation of the overall patterns of genetic (co)variance of size during growth under different conditions. However, specific hypotheses regarding genetic covariance across stage and environment, which are based on visual inspection of V_a in the model 1 **G** (table 1), can be directly tested by further analyzing the data as a G × E interaction (Lynch and Walsh 1998). In order to determine whether the environment-dependent changes in genetic variance observed in table 1 are statistically significant, we tested for a significant three-way interaction between sire (the genetic effect) and the two sources of environmental variation (time and treatment). A significant interaction between sire and either of the other factors would indicate that the variance among sires depends on the environment. As before, the two measures of body size, pronotum width and mass, were analyzed in a multivariate fashion.

The mixed model for each sex was

$$\begin{aligned}\mathbf{Y}_{ijkln} = {} & \boldsymbol{\mu} + \mathbf{S}_i + \mathbf{D}_{j(i)} + \mathbf{H}_k + \mathbf{F}_l + \mathbf{T}_m \\ & + \mathbf{F}_l \times \mathbf{T}_m + \mathbf{S} \times \mathbf{F}_{il} + \mathbf{S} \times \mathbf{T}_{im} \\ & + (\mathbf{S} \times \mathbf{T} \times \mathbf{F})_{ilm} + \boldsymbol{\epsilon}_{ijklmn}.\end{aligned} \tag{2}$$

As in model 1, hatching day (**H**), food treatment (**F**), and time period (**T**) were treated as fixed effects, and sire (**S**) and dam nested within sire (**D**) are random effects. The interaction between the sire component, time of measurement, and food treatment equates to a test of whether the effect of time on the sire variance depends upon the food treatment, in a fashion exactly analogous to testing for a G × E interaction when different genotypes are allowed to

develop in different environments (Lynch and Walsh 1998, p. 667). As in model 1, all random effects (sire, sire × food treatment, sire × time, and sire × time × food treatment) were fitted by a factor analytic covariance structure with two factors (the maximum number of factors for this two-trait model). As in model 1, both the first two factors were included in the analysis.

Results

Larval Growth and Survival

Mean body size (PC1) of larvae from the low-food treatment was substantially smaller than those in the control treatment (fig. 1). For both males and females, this effect was significant (food [F] effect: males, $F_{1,372} = 412.16$, $P < .001$, females, $F_{1,337} = 385.25$, $P < .001$; hatch day [H] effect: males, $F_{1,372} = 19.23$, $P < .001$, females, $F_{1,337} = 9.44$, $P = .002$; F × H: males, $F_{1,372} = 3.20$, $P = .075$, females, $F_{1,337} = 1.29$, $P = .256$). By emergence to adult, individuals in the low-food treatment had caught up to and even surpassed the body size of individuals in the control treatment (fig. 1). The larger size of the low-food individuals, though small, was significant for females and approached significance in males (food effect: males, $F_{1,384} = 3.39$, $P = .071$, females, $F_{1,349} = 4.78$, $P < .001$; hatch day effect: males, $F_{1,384} = 0.01$, $P = .929$, females, $F_{1,349} = 4.41$, $P = .036$; F × H: males, $F_{1,384} = 3.77$, $P = .053$, females, $F_{1,349} = 0.03$, $P = .855$). To confirm that this represents significantly accelerated (compensatory) growth in the low-food treatment, we tested for treatment effects on the difference in body size measured in the larvae and the adult. This analysis demonstrated that growth was significantly accelerated in the low-food treatment in both males and females (food effect: males, $F_{1,372} = 374.98$, $P < .001$, females, $F_{1,337} = 306.23$, $P < .001$; hatch day effect: males, $F_{1,372} = 14.53$, $P < .001$, females, $F_{1,337} = 1.96$, $P = .162$; F × H: males, $F_{1,372} = 0.28$, $P = .600$, females, $F = 0.93_{1,337}$, $P = .336$).

Development to the fourth (final instar) in the low-food treatment was delayed in both males and females by about 1 day (male: $H = 7.07$ days, $L = 8.10$ days; females, $H = 7.12$ days, $L = 7.98$ days). This treatment effect was significant in both sexes (food effect: males, $F_{1,384} = 187.43$, $P < .001$, females, $F_{1,349} = 108.60$, $P < .001$; hatch day effect: males, $F_{1,384} = 20.17$, $P < .001$, females, $F_{1,349} = 12.70$, $P < .001$; F × H: males, $F_{1,384} = 3.05$, $P = .081$, females, $F_{1,349} = 3.50$, $P = .062$). In contrast, development from fourth instar to adult, when food levels did not differ between treatments, differed little between treatments for either sex (male, $H = 10.27$ days, $L = 10.23$ days; females, $H = 10.34$ days, $L = 10.23$ days). These differences were not significant (food effect: males, $F_{1,384} = 0.41$, $P = .522$, females, $F_{1,349} = 1.83$, $P = .177$; hatch day effect: males, $F_{1,384} = 5.51$, $P = .019$, females, $F_{1,349} = 0.12$, $P = .719$; F × H: males, $F_{1,384} = 0.18$, $P = .674$, females, $F_{1,349} = 0.39$, $P = .533$). These analyses

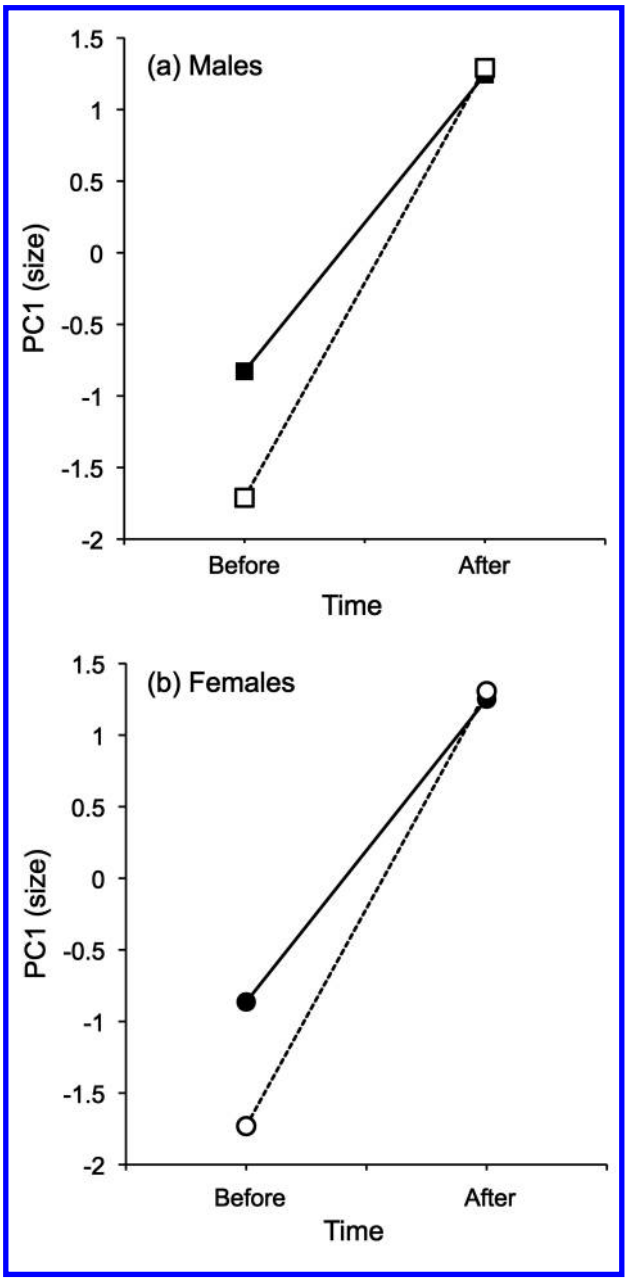

Figure 1: Effect of food treatment on body size (principal component 1 for body mass and pronotum width) at two points in development (early, at the end of the food manipulation) and at eclosion to the adult stage for males (*a*) and females (*b*). Solid symbols and lines denote the high-food treatment, and open symbols and broken lines represent the low-food treatment. Standard error bars are smaller than the symbols.

Table 2: Eigenvalues and eigenvectors for first two factors of male and female **G**

		Eigenvector							
		High food				Low food			
Sex, factor	Eigenvalue	PW early	PW late	Mass early	Mass late	PW early	PW late	Mass early	Mass late
Males:									
1	.558*	.339	.233	.325	.18	.527	.442	.086	.46
2	.102	−.281	.351	−.231	.105	−.438	.373	.607	.183
Females:									
1	.313[a]	.005	.001	−.18	−.041	.625	.536	−.073	.531
2	.165	.923	.157	−.133	.251	.107	−.141	−.098	−.031

Note: PW = pronotum width.

[a] In females, the first genetic factor only approached significance ($P < .13$).

* The genetic factor specified accounted for a significant amount of genetic variance ($P < .0001$).

demonstrate that the increased rate of body size gain in the low-food treatment during this period of compensatory growth cannot be attributed to an extended period of development.

Mortality rates were calculated from 805 larvae of the original 822, because 17 larvae escaped their petri dishes or were damaged during handling (11 in low food and 6 in high food). A total of 741 (92%) individuals survived to maturity. Mortality during the early period when food differed between treatments (days 1–7) was $N = 7$ for high food and $N = 24$ for low food, which is a significant difference ($\chi^2 = 9.34$, df = 1, $P = .002$). During the fourth instar and pupation, when food levels did not differ between treatments, 17 and 16 individuals in the high and low treatments died ($\chi^2 = 0.03$, df = 1, $P > .9$). These data suggest that the low-food treatment appears to have induced stress; however, mortality rates were elevated only during the period of resource restriction and not in the subsequent period of accelerated growth.

Genetic Variance in Size: Effects of Developmental Stage and Environment

Initial analyses indicated that a model allowing an unconstrained covariance structure at the sire level would not converge. We therefore fit a series of reduced-rank models at the sire level to determine the number of significant genetic factors that were sufficient to explain the estimated genetic variance. The first genetic factor explained a significant amount of the variance in males (males: $\chi^2 = 39.9$, df = 8, $P < .0001$) but only approached significance in females ($\chi^2 = 12.3$, df = 8, $P = .13$). In males, this first genetic factor accounted for nearly twice as much genetic variance as in females, respectively (tables 1, 2). The second genetic factor was also tested for each sex, but neither explained a significant amount of variance ($\chi^2 = 8.9$, df = 7, $P = .38$ and $\chi^2 = 7.5$, df = 7, $P = .26$ for males and females, respectively). However, to minimize bias in the estimates of the eigenvectors that may result from using too few factors, the **G** matrix was based on the first two genetic factors as suggested by Meyer and Kirkpatrick (2008).

Visual inspection of the resulting **G** matrices for the first two genetic factors (table 1) suggested that the change in genetic variance over ontogeny appeared to depend on food treatment. Beginning with **G** for males (upper part of table), genetic variance in mass decreased over time (early to late) in the high-food treatment, while in the low-food treatment, there was a substantial increase in genetic variance for mass over time. For pronotum width, genetic variance decreased over ontogeny in the high-food treatment, as was the case for mass. At low food, genetic variance in pronotum also with decreased with time, as it did at high food, but this decrease was proportionally smaller than observed in high food.

In females, the first genetic factor did not explain a large (or significant) proportion of the variance, and the genetic variances and covariances were correspondingly small. Nevertheless, the ontogenetic pattern in variances observed in females is qualitatively similar to that observed in males.

Notably, the genetic covariances across environments are in large part opposite in sign for females and males. For each trait-by-time combination, covariances between the high- and low-food treatments are almost uniformly positive in males. In females, these covariances are most often negative, although all values were very close to 0.

Hypothesis Test

From the G × E analysis, we were able to directly test the hypothesis that food treatment, time, and their interaction had significant effects on the amount of genetic variance present in size (table 3). In males, we found a highly significant effect of the interaction between sire, treatment, and time. These results suggest that the amount of genetic

Table 3: Hypothesis test for effects of sire, time, and treatment on body size

Sex, source	Eigenvalue	Eigenvector		χ^2	df	*P*
		PW	Mass			
Males:						
Sire	.063	.568	.823	2.4	2	.30
Sire × treatment	.040	.100	.995	3.2	2	.20
Sire × time	.008	.883	.469	2.0	2	.37
Sire × treatment × time	.050	.842	−.539	13.8	2	.001
Females:						
Sire	.000	.000	1.000	0	2	1.00
Sire × treatment	.021	.251	.968	.7	2	.70
Sire × time	.018	−.611	.792	1.6	2	.45
Sire × treatment × time	.039	−.164	.986	2.4	2	.30

Note: PW = pronotum width.

variance changed over ontogeny, but the direction/magnitude of change depended on the growth environment. This analysis provides strong statistical support for our earlier interpretation of the **G** matrix (table 2), which suggests that changes in genetic variance during ontogeny depend on the food treatment. In males, there was no significant sire effect or effects of its interaction with time or treatment.

In females, we found no significant effects of sire, its interactions with time and treatment, or the three-way interaction. These results reflect the fact that very little genetic variance was detected in females (table 2).

Discussion

Our study provides the first experimental evidence demonstrating that ontogenetic patterns in genetic variance in size in animals are influenced by resource conditions experienced during development. In males, a substantial amount of genetic variance was present in pronotum width and mass in all time-by-treatment combinations, including the period of environmentally induced compensatory growth. However, the pattern of change in this variance over ontogeny differed in the two food treatments. Genetic variance in both measures of size (mass and pronotum width) tended to decrease late in ontogeny in high food. However, in the low-food treatment, this convergent effect was reduced for pronotum width, and for mass, variance actually increased late in ontogeny. Although ontogenetic patterns in size variance were similar in females, we could not detect significant genetic variance in these size measures, possibly because only a small proportion of total phenotypic variance is attributable to additive genetic variance. The growth environment effects that were detectable in males may account for the discrepancy seen in lab and field studies of age effect on the genetic variances in a variety of traits.

Sex Differences in Genetic Variance for Growth

Sex differences in genetic variability observed here are not unexpected, given that males and females experience different selective pressures on life-history traits, including growth rate and body size (e.g., Andersson 1994). Males tended to be much more genetically variable than females at both stages of ontogeny and in both food treatments (tables 1, 2). Moreover, sires that attained relatively large sizes at one time and environment tended to do so in all environments (tables 1, 2). This suggests that those genetic attributes that lead to large size of males tend to be consistent among stage in ontogeny and food environment. Our analysis indicated that in contrast to males the cross-environmental covariance was negative in females (tables 1, 2). Despite the lack of statistical support for these patterns in females, they nevertheless suggest an intriguing possibility, which is that female genotypes that achieve a large body size at high food are less well equipped to reach a large body size at low food and that the underlying genetics of female growth trajectories may differ from males. However, larger data sets may allow this pattern to be explored directly by analysis of between-sex genetic covariances.

The Ontogeny of Genetic Variance in Size in High- and Low-Food Regimes in Males

In the high-food regime, genetic variance in mass and pronotum width tended to decrease from the beginning of the fourth instar to maturation in both sexes (table 1). These results are consistent with the early laboratory studies on growth in rodents (e.g., Cheverud et al. 1983; Atchley 1984; Riska et al. 1984) and also in the burying beetle *Nicrophorus pustulatus* (Rauter and Moore 2002). Riska et al. (1984) referred to this pattern as convergent growth, although earlier authors referred to the same phenotypic pattern as compensatory growth (Monteiro and Falconer

1966). We will refer to the decline in genetic variance over a period of growth as convergent growth and reserve the term "compensatory growth" to refer to the acceleration of growth following a period of growth restriction that is environmentally induced.

In convergent growth, genotypes that grow fast early in life tend to grow slowly late in life and vice versa. Atchley (1984) argued that this pattern suggests an endogenous regulation of growth, where growth is modulated with respect to size rather than to age. One explanation for convergent growth is that selection on size at maturity is strong relative to selection on sizes at earlier juvenile stages, resulting in canalization of the size at maturation (Cheverud et al. 1983; Riska et al. 1984; Metcalfe and Monaghan 2001; Flatt 2005). Size dependence of reproductive performance is common in adults of both sexes, and in many species the scope for adjustment of postmaturation body size is limited. In determinate growers, structural size is fixed at maturity, and in indeterminate growers, any investment to somatic growth comes with some direct cost to reproductive investment. Nevertheless, it is possible that selection on body size at maturity could be lower in indeterminate growers such as fish, which could lead to taxon-specific differences in ontogenetic patterns in genetic variance.

In contrast to the high-food treatment, genetic variance in mass tended to increase late in ontogeny in the low-food treatment (table 2) when mean growth rate was accelerated (fig. 1). Divergent growth at the genetic level has been observed in many species (Lytle 2001), though we believe this is the first time that a switch from convergent to divergent growth can be attributed to a specific environmental variable. There are three possible processes that may account for the observed environmentally determined switch from convergent to divergent growth late in ontogeny. First, growth may have been genetically divergent in both environments, but selection-induced mortality on fast or slow growers obscures this in the high-food environment. This is unlikely to be a general explanation since selection is expected to be stronger in the low-food stressful environment than in the high-food environment. Furthermore, we can eliminate this hypothesis for this experiment because mortality was considerably higher in the low-food treatment.

A second possibility is that in the low-food treatment, reduced ration early in ontogeny or the induced accelerated growth late in ontogeny acted as a stress that exposed hidden genetic variance, and this overwhelmed any buffering mechanisms that would otherwise canalize size variance. The higher mortalities observed at low food in our experiments support the view that low food was stressful. Earlier similar experiments have demonstrated that this sort of compensatory growth treatment induces long-term costs (Dmitriew and Rowe 2007). This hypothesis is in accord with the view that otherwise hidden or "cryptic" genetic variance may be exposed when organisms encounter stress or novel environments (e.g., Hoffman and Parsons 1991; Houle 1992; Flatt 2005). It has been suggested that the buildup of cryptic genetic variation determines to some extent the ability of animals to evolve; while many of the accumulated mutations that contribute to the cryptic genetic variation may have deleterious effects, they also provide the raw material for natural selection to act on (e.g., Flatt 2005). Genetic variation is necessary for animals to adapt to novel or stressful conditions, and the accumulated cryptic variation may be expected in species such as *Harmonia axyridis*, which have an evolutionary history of variable food supplies as well as a considerable ability to exploit novel environments (Koch 2003).

Finally, it is possible that individuals in the low-food treatment are chasing a target adult body size that is unattainable, or nearly so, under conditions of food stress. The effect of this would be to exaggerate the genotypic differences in maximal growth rate, as we observed in the low-food treatment. Following a period of food restriction that depresses growth, not all genotypes may be capable of growing fast enough to reach the target adult size. By contrast, under high-food conditions, a greater range of growth genotypes would be capable of achieving the target. The effect of this would be a contraction of genetic variance in size between the fourth instar and eclosion in the high-food treatment but not in the low-food treatment, just as we observed (table 1).

The environment dependence of ontogenetic patterns in genetic variance shown here suggests an explanation for the lack of consistency among the results of published studies on wild and laboratory populations (Réale et al. 1999; Charmantier and Garant 2005; Wilson et al. 2005). In the wild, resource availability and other sources of stress may be extremely variable over the period of development and among individuals; thus, the interaction between genotype and environment may result in the expression of a large amount of genetic variation in growth, which cannot be canalized, as is the case in more benign laboratory environments. To our knowledge, only one previous study combined the measurement of ontogenetic change in genetic variance with an environmental manipulation. In a study of the effects of parental care in burying beetles, Rauter and Moore (2002) found that the coefficient of genetic variance in body mass declined between the early larval stage and eclosion regardless of whether care was provided. Burying beetles receiving parental care tend to be in higher condition, though it is possible that its absence is insufficient to expose cryptic genetic variance.

Conclusion

We have shown that there is substantial variation in male body size traits in all time-by-treatment combinations but that the direction of change in genetic variation in mass over ontogeny was dependent on the food environment. We detected some intriguing similarities between the sexes but could not detect significant genetic variance in females, which hampered our exploration of these effects. Our approach to the genetic analysis of growth in this article has been to use the expression of size-related traits in different environments and at different stages of ontogeny as essentially different traits within the classic G × E framework (Lynch and Walsh 1998). An alternative powerful approach that shows great promise is to model the reaction norms across ontogeny using random regression approaches (Kirkpatrick et al. 1990; Kirkpatrick and Lofsvold 1992; Meyer and Hill 1997; Kingsolver et al. 2001; Griswold et al. 2008). Using this approach, which requires several repeated measures of a trait, genetic variance for a trait could be directly estimated as the variance around the reaction norms (e.g., growth rate under different resource levels) at the sire level. Further exploration of the patterns observed here and the apparent difference among the sexes would profit from employing this promising alternative approach.

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Literature Cited

Ali, M., A. Nicieza, and R. J. Wootton. 2003. Compensatory growth in fishes: a response to growth depression. Fish and Fisheries 4: 147–190.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Arendt, J. D. 1997. Adaptive intrinsic growth rates: an integration across taxa. Quarterly Review of Biology 72:149–177.

Atchley, W. R. 1984. Ontogeny, timing of development, and genetic variance-covariance structure. American Naturalist 123:519–540.

Atchley, W. R., and J. Zhu. 1997. Developmental quantitative genetics, conditional epigenetic variability and growth in mice. Genetics 147:765–776.

Charlesworth, B. 1994. Evolution in age-structured populations. 2nd ed. Cambridge University Press, Cambridge.

Charmantier, A., and D. Garant. 2005. Environmental quality and evolutionary potential: lessons from wild populations. Proceedings of the Royal Society B: Biological Sciences 272:1415–1425.

Charmantier, A., C. Perrins, R. H. McCleery, and B. C. Sheldon. 2006. Age-dependent genetic variance in a life-history trait in the mute swan. Proceedings of the Royal Society B: Biological Sciences 273:225–232.

Cheverud, J. M., J. J. Rutledge, and W. R. Atchley. 1983. Quantitative genetics of development: genetic correlations among age-specific trait values and the evolution of ontogeny. Evolution 37:895–905.

Dmitriew, C., and L. Rowe. 2005. Resource limitation, predation risk and compensatory growth in a damselfly. Oecologia (Berlin) 142: 150–154.

———. 2007. Effects of early resource limitation and compensatory growth on lifetime fitness in the ladybird beetles (*Harmonia axyridis*). Journal of Evolutionary Biology 20:1298–1310.

Dworkin, I. 2005. Canalization, cryptic variation and developmental buffering: a critical examination and analytical perspective. Pages 131–158 *in* B. Hallgrimsson and B. K. Hall, eds. Variation. Academic Press, New York.

Falconer, D. S., and T. F. C. MacKay. 1996. Introduction to quantitative genetics. 4th ed. Longman, Harlow, Essex.

Flatt, T. 2005. The evolutionary genetics of canalization. Quarterly Review of Biology 80:287–317.

Fraser, D. J., L. K. Weir, T. L. Darwish, J. D. Eddington, and H. A. Hutchings. 2007. Divergent compensatory growth responses within species: linked to contrasting migrations in salmon? Oecologia (Berlin) 153:543–553.

Griswold, C., R. Gomulkiewicz, and N. Heckman. 2008. Hypothesis testing in comparative and experimental studies of function-valued traits. Evolution 62:1229–1242.

Hine, E., and M. W. Blows. 2006. Determining the effective dimensionality of the genetic variance-covariance matrix. Genetics 173: 1135–1144.

Hoffmann, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends in Ecology & Evolution 14:96–101.

Hoffmann, A. A., and P. A. Parsons. 1991. Evolutionary genetics and environmental stress. Oxford University Press, Oxford.

Houle, D. 1992. Comparing evolvability and variability of quantitative traits. Genetics 130:195–204.

———. 1998. How should we explain variation in the genetic variance of traits? Genetica 102/103:241–253.

Hughes, K. A., J. A. Alipaz, J. M. Drnevich, and R. M. Reynolds. 2002. A test of evolutionary theories of aging. Proceedings of the National Academy of Sciences of the USA 99:14286–14291.

Kingsolver, J. G., R. Gomulkiewicz, and P. A. Carter. 2001. Variation, selection and evolution of function-valued traits. Genetica 112:87–114.

Kirkpatrick, M., and D. Lofsvold. 1992. Measuring selection and constraint in the evolution of growth. Evolution 46:954–971.

Kirkpatrick, M., and K. Meyer. 2004. Direct estimation of genetic principal components: simplified analysis of complex phenotypes. Genetics 168:2295–2306.

Kirkpatrick, M., D. Lofsvold, and M. Bulmer. 1990. Analysis of the inheritance, selection and evolution of growth trajectories. Genetics 124:979–993.

Koch, R. L. 2003. The multicoloured Asian lady beetle *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. Journal of Insect Science 3:1–16.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

Lytle, D. A. 2001. Convergent growth regulation in arthropods: biological fact or statistical artifact? Oecologia (Berlin) 128:56–61.

Mangel, S. B., and M. Munch. 2005. A life-history perspective on short- and long-term consequences of compensatory growth. American Naturalist 166:E155–E176.

Medawar, P. B. 1952. An unsolved problem of biology. Lewis, London.
Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology & Evolution 16:254–260.
Meyer, K., and W. G. Hill. 1997. Estimation of genetic and phenotypic covariance functions for longitudinal or "repeated" records by restricted maximum likelihood. Livestock Production Science 47: 185–200.
Meyer, K. and M. Kirkpatrick. 2008. Perils of parsimony: properties of reduced-rank estimates of genetic covariance matrices. Genetics 180:1153–1166.
Monteiro, L. A., and D. S. Falconer. 1966. Compensatory growth and sexual maturity in mice. Animal Production 8:179–192.
Price, T. D., and D. Schluter. 1991. On the low heritability of life history traits. Evolution 45:853–861.
Promislow, D. E. L., M. Tatar, A. A. Khazaeli, and J. W. Curtsinger. 1996. Age-specific patterns of genetic variance in *Drosophila melanogaster*. 1. Mortality. Genetics 143:839–848.
Rauter, C. M., and A. J. Moore. 2002. Quantitative genetics of growth and development time in the burying beetle *Nicrophorus pustulatus* in the presence and absence of post-hatching parental care. Evolution 56:96–110.
Réale, D., M. Festa-Bianchet, and J. T. Jorgenson. 1999. Heritability of body mass varies with age season in wild bighorn sheep. Heredity 83:526–532.
Riska, B., W. R. Atchley, and J. J. Rutledge. 1984. A genetic analysis of targeted growth in mice. Genetics 107:79–101.
Roff, D. A. 1992. The evolution of life histories: theory and analysis. Chapman & Hall, New York.
———. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. Journal of Evolutionary Biology 13:434–445.
SAS Institute. 2008. SAS software, version 9.2. SAS Institute, Cary, NC.
Snoke, M. S., and D. E. L. Promislow. 2003. Quantitative genetic tests of recent senescence theory: age-specific mortality and male fertility in *Drosophila melanogaster*. Heredity 91:546–556.
Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.
Stearns, S. C., and T. J. Kawecki. 1994. Fitness sensitivity and the canalization of life-history traits. Evolution 48:1438–1450.
Stevens, D. J., M. Hansell, and P. Monaghan. 1999. Developmental trade-offs and life histories: strategic allocation of resources in caddis flies. Proceedings of the Royal Society B: Biological Sciences 267:1511–1515.
Strobbe, F., and R. Stoks. 2004. Life history reaction norms to time constraints in a damselfly: differential effects on size and mass. Functional Ecology 83:187–196.
Tanner, J. M. 1963. Regulation of growth in size in mammals. Nature 199:845–850.
Tatar, M., D. E. L. Promislow, A. Khazaeli, and J. Curtsinger. 1996. Age-specific patterns of genetic variance in *Drosophila melanogaster*. II. Fecundity and its genetic correlation with age-specific mortality. Genetics 143L:849–858.
Wilson, A. J., and D. Réale. 2006. Ontogeny of additive and maternal effects: lessons from domestic mammals. American Naturalist 167: E23–E38.
Wilson, A. J., L. E. B. Kruuk, and D. W. Coltman. 2005. Ontogenetic patterns in heritable variation for body size: using random regression models in a wild ungulate population. American Naturalist 166:E177–E192.

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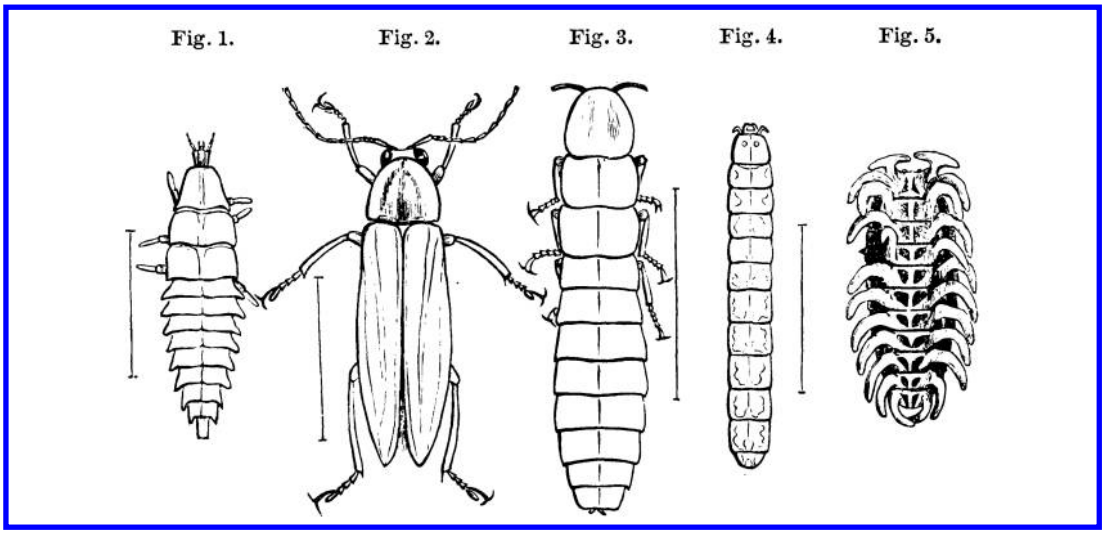

Fig. 1 is, very probably, the larva of a genus allied to *Photuris*, of which *P. Pensylvanica* (Fig. 2) is the adult male. We found this larva early in May, under a stone in damp ground, at Swampscot, Mass. Fig. 3 is an adult female glowworn from Zanzibar. Fig. 4 is a truly luminous larva … found at Roxbury, Mass. Fig. 5 pictures a most singular larva … found by Rev. E. C. Bolles at Westbrook, Maine, under leaves. From "The Cucuyo; or, West Indian Beetle" by G. A. Perkins, M.D. (*American Naturalist*, 1868, 2:422–433).